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Article

$G\alpha_{o/i}$ and $G\alpha_s$ Signaling Function in Parallel with the MSP/Eph Receptor to Control Meiotic Diapause in *C. elegans*

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Summary

Background: A conserved biological feature of sexual reproduction in animals is that oocytes arrest in meiotic prophase and resume meiosis in response to extraovarian signals. In *C. elegans*, sperm trigger meiotic resumption by means of the major sperm protein (MSP) signal. MSP promotes meiotic resumption by functioning as an ephrin-signaling antagonist and by counteracting inhibitory inputs from the somatic gonadal sheath cells.

Results: By using a genome-wide RNAi screen in a female-sterile genetic background, we identified 17 conserved genes that maintain meiotic arrest in the absence of the MSP signal. In vitro binding experiments show that MSP promotes oocyte mitogen-activated protein kinase activation and meiotic maturation in part through direct interaction with the VAB-1 Eph receptor. Four conserved proteins, including a disabled protein (DAB-1), a vav family GEF (VAV-1), a protein kinase C (PKC-1), and a STAM homolog (PQN-19), function with the VAB-1 Eph/MSP receptor in oocytes. We show that antagonistic $G\alpha_{o/i}$ and $G\alpha_s$ signaling pathways function in the soma to regulate meiotic maturation in parallel to the VAB-1 pathway. $G\alpha_s$ activity is necessary and sufficient to promote meiotic maturation, which it does in part by antagonizing inhibitory sheath/oocyte gap-junctional communication.

Conclusions: Our findings show that oocyte Eph receptor and somatic cell G protein signaling pathways control meiotic diapause in *C. elegans*, highlighting contrasts and parallels between MSP signaling in *C. elegans* and luteinizing hormone signaling in mammals.

Introduction

Oocytes of most sexually reproducing animals arrest in meiotic prophase and actively maintain their arrest for prolonged periods—up to 50 years in humans. In response to hormonal signaling, oocytes resume meiosis in the highly conserved process of meiotic maturation, which prepares the oocyte for fertilization [1, 2]. Oocyte meiotic maturation is defined by the transition between diakinesis and metaphase of meiosis I and is accompanied by nuclear envelope breakdown, cortical cytoskeletal rearrangement, meiotic spindle assembly, and chromosome congression. Chromosome missegregation in female meiosis I represents the leading cause of human birth defects (e.g., Down syndrome). Because advanced maternal age is the most significant risk factor [3], the mechanisms that maintain meiotic diapause and preserve oocyte vitality are of intense interest.

Great strides have been made in understanding the control of cell-cycle progression during the meiotic maturation process, culminating in the discovery of the maturation promoting factor (Cdk1/cyclin B; [4]). Mitogen-activated protein kinase (MAPK) cascades also play an important role in controlling meiotic progression [5]. By contrast, comparatively less information is available about the intercellular signaling pathways that regulate meiotic resumption. Unifying conclusions from studies in vertebrate and invertebrate systems are that soma-germline interactions play a crucial role and that regulation involves both positively and negatively acting pathways [1]. As meiotic maturation signals have been characterized in several invertebrate systems, studies in these organisms may offer both comparative and mechanistic insights.

In *C. elegans*, sperm export the major sperm protein (MSP) by a vesicle-budding mechanism to trigger oocyte MAPK activation and meiotic maturation (Figure 1A; [6–8]). MSP is also the key cytoskeletal element required for the actin-independent amoeboid locomotion of nematode spermatozoa [9]. Since hermaphrodites produce only a fixed number of sperm, meiotic maturation rates are initially high for the first 2 days of adulthood but decline as sperm are used for fertilization and the MSP signal disappears [8, 10]. Similarly, in sex-determination mutants of *C. elegans*, which fully feminize the hermaphrodite gonad (e.g., *fog-2* or *fog-3*), oocytes arrest until sperm are supplied by mating. In *C. elegans*, the vital processes of meiotic maturation and ovulation are tightly coupled to sperm availability through a complex regulatory network involving both negative and positive controls. Parallel genetic pathways defined by *vab-1*, which encodes an ephrin receptor, and *ceh-18*, which encodes a POU-homeoprotein expressed in gonadal sheath cells but not oocytes, together compose an MSP-sensing control mechanism that inhibits meiotic maturation, MAPK activation, and ovulation when sperm are not present in the reproductive tract (Figure 1A; [7]). Negative regulators of meiotic maturation, such as *vab-1* and *ceh-18*, are identified by RNAi knockdown or loss-of-function mutations that cause females to mature oocytes in the absence of the MSP signal. In contrast, positive regulators, such as *oma-1* and *oma-2*, which encode two TIS-11 zinc finger proteins expressed in the germline [11], are identified by RNAi knockdown or loss-of-function mutations that reduce or block meiotic maturation in hermaphrodites in the presence of the MSP signal.

Here we report the results of a comprehensive RNAi screen undertaken to identify regulators of meiotic

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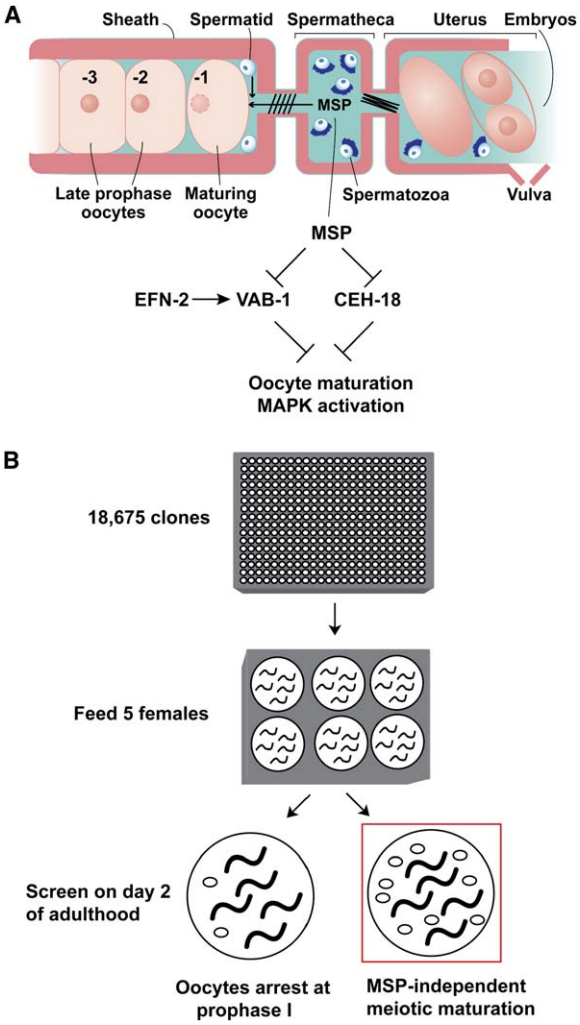

Figure 1. A Genome-Wide RNAi Screen for Negative Regulators of Oocyte Meiotic Maturation

(A) Oocytes undergo meiotic maturation in an assembly-line fashion in response to MSP signaling. The oocyte VAB-1 Eph receptor pathway and a sheath cell pathway, defined by CEH-18, negatively regulate oocyte MAPK activation and meiotic maturation. MSP antagonizes these inhibitory inputs to promote meiotic maturation.

(B) Flowchart for the genome-wide RNAi screen in a *fog-2(q71)* female-sterile background. Most clones have no effect on meiotic arrest (bottom left), whereas RNAi of 17 clones results in MSP-independent meiotic maturation without disrupting gonadal morphology (bottom right).

diapause in the absence of the MSP signal. This genome-wide RNAi screen identified 16 new negative regulators of meiotic maturation. Four conserved proteins, DAB-1, PQN-19, PKC-1, and VAV-1, function with the VAB-1 MSP/Eph receptor in oocytes. In parallel to the VAB-1 MSP/Eph receptor pathway, antagonistic $G\alpha_{o/i}$ and $G\alpha_s$ signaling pathways define negatively and positively acting somatic cell inputs, respectively. $G\alpha_s$ signaling is necessary and sufficient to trigger oocyte MAPK activation and meiotic maturation, which it does in part by antagonizing inhibitory sheath/oocyte gap-junctional communication. This finding, together with the results from mammalian systems [12, 13], suggest that the involvement of the $G\alpha_s$ pathway may be an ancestral feature of meiotic maturation signaling.

Results and Discussion

Identification of Negative Regulators of MSP Signaling via a Genome-Wide RNAi Screen

MSP promotes oocyte meiotic maturation by antagonizing two parallel negative regulatory circuits: an oocyte VAB-1 Eph receptor pathway and a somatic gonadal sheath cell pathway defined by the POU-homeoprotein CEH-18 (Figure 1A; [7]). We reasoned that additional components of the VAB-1 and CEH-18 pathways were likely to function as negative regulators, and we sought to identify them by a genome-wide RNAi screen (Figure 1B). We performed this screen with a *fog-2(q71)* female sterile strain in which oocytes arrest at prophase of meiosis I and are retained in the gonad arm due to the absence of MSP. We screened for rare RNAi clones in which meiotic maturation and ovulation occur at elevated rates and unfertilized oocytes are laid onto the bacterial lawn in increased numbers despite the absence of MSP (Figure 1B and Table 1).

We identified 175 clones that fell into two categories depending on their consequence for gonadal structure: class I (17 clones) had no appreciable effects, and class II (158 clones) caused defects in gonadal morphology. The disruption of gonadal integrity observed after RNAi of class II clones limits our ability to study their roles in MSP signaling to varying degrees. Thus, here we focus on the 17 class I-positive clones (Table 1). Class I genes encode several proteins with well-characterized intercellular signaling functions, such as components of multiple G protein signaling pathways (*goa-1*, *kin-2*, *gpb-1*, and *gsa-1*), protein kinase Cε (*pkc-1*), a 14-3-3 protein (*par-5*), and a disabled homolog (*dab-1*). RNAi to class I genes had no apparent effects on germline sex determination, yet we verified the absence of MSP by immunostaining in all cases (Table 1). The fact that *ceh-18* was identified validates the rationale and effectiveness of the screen. This screen did not identify *vab-1*, *nmr-1*, and *itr-1*, three known negative regulators of meiotic maturation [7, 14]. Within class I, the level of derepression of meiotic maturation differed between clones (Table 1). Remarkably, RNAi of *goa-1* and *kin-2* resulted in approximately 60% of the wild-type rate in the presence of sperm. MSP is sufficient to activate MAPK in female gonads when assessed with antibodies to the diphosphorylated activated form of MPK-1 MAPK (MAPK-YT; [6]). RNAi to six genes (*goa-1*, *kin-2*, *gpb-1*, *inx-14*, *inx-22*, and *ptc-1*) resulted in MAPK activation in the absence of MSP (Table 1 and Figure 2). These results thus define new negative regulators of the meiotic maturation process and highlight the complexity of the signaling pathways involved.

Germline and Somatic Pathways Regulate Meiotic Maturation

The somatic cells surrounding the oocyte play a key role in negatively regulating meiotic progression in mammals and *C. elegans* [7, 15]. In *C. elegans*, the gonadal sheath cells form gap junctions with oocytes, and these gap junctions are rare or absent in *ceh-18* mutants [16, 17], suggesting their importance. Gap-junctional communication between sheath cells and oocytes are a critical aspect of the negative control of meiotic maturation, as shown by the fact that our screen identified two innexin

Table 1. Negative Regulators of Meiotic Maturation Identified in a Genome-Wide RNAi Screen

Gene[a]	Description	MSP[b]	Oocyte Maturation Rate in Females (hr^{-1} gonad arm^{-1})[c]	MAPK Activation[d]
Control	Wild-type hermaphrodite	+	2.50 ± 0.41 (17)	25/25 (+)
Control[e]	*fog-2(q71)* unmated female	–	0.16 ± 0.10 (17)	1/20 (–)
Control	*fog-2(q71)* mated female	+	2.42 ± 0.35 (14)	18/18 (+)
goa-1	Heterotrimeric $G_{o/i}$ α protein subunit	–	1.67 ± 0.38 (36)[f]	14/15 (+)
kin-2	cAMP-dependent protein kinase (regulatory subunit)	–	1.50 ± 0.30 (9)[f]	9/13 (+)
gpb-1	Heterotrimeric G_{β} protein subunit	–	0.88 ± 0.24 (21)[f]	13/18 (+)
gsa-1	Heterotrimeric $G_s\alpha$ protein subunit	–	0.37 ± 0.18 (12)[g]	1/19 (–)
rpt-3	Component of 26S proteasome[h]	–	0.44 ± 0.15 (25)[f]	1/14 (–)
inx-14	Gap junction protein (innexin family)	–	0.99 ± 0.30 (21)[f]	17/17 (+)
inx-22	Gap junction protein (innexin family)	–	0.90 ± 0.36 (30)[f]	15/18 (+)
ran-1	Ran GTPase	–	0.72 ± 0.13 (6)[f]	0/18
ceh-18	POU-Homeo domain transcription factor	–	0.45 ± 0.18 (12)[f,i]	1/17 (+)
arf-1.1	Arf-family GTP binding protein	–	0.64 ± 0.28 (10)[f]	1/19 (–)
ptc-1	Patched receptor	–	0.60 ± 0.27 (18)[f,j]	12/15 (+)
phi-11	Splicing factor 3B, subunit 1[k]	–	0.50 ± 0.15 (10)[f]	0/15
par-5	Encodes 14-3-3 protein	–	0.49 ± 0.20 (28)[f]	0/17
pqn-19	Signal-transducing adaptor molecule (STAM)	–	0.48 ± 0.34 (12)[g]	0/17
pkc-1	Protein kinase C	–	0.44 ± 0.11 (9)[f]	1/17 (–)
vav-1	Vav-GEF proto-oncogene homolog	–	0.42 ± 0.13 (15)[f]	0/16
dab-1	Disabled homolog	–	0.38 ± 0.14 (15)[f]	0/16

[a] Shown are class I positive clones, RNAi of which does not appreciably alter gonadal morphology. The identity of the clones was verified by DNA sequencing.
[b] The absence of MSP in unmated female gonads was confirmed by staining with monoclonal anti-MSP antibodies.
[c] Oocyte maturation rates are expressed as the number of maturations per gonad arm per hour and were measured in 2-day-old adult *fog-2(q71)* females (excepting the wild-type hermaphrodite control). The number of worms scored is given in the parentheses.
[d] The fraction of gonad arms showing MAPK-YT staining. MAPK activation was further classified according to whether the observed staining was strong or weak, indicated by (+) or (–), respectively.
[e] Mock RNAi with the empty vector, L4440, served as a control.
[f] $p < 0.001$ compared to *control(RNAi)* in *fog-2(q71)* females.
[g] $p < 0.01$ compared to *control(RNAi)* in *fog-2(q71)* females.
[h] RNAi of many 26S proteasome components resulted in gonadal defects and scored as class II positives in the screen.
[i] *ceh-18(mg57);fog-2(q71)* females have a maturation rate of 0.75 ± 0.32 [7].
[j] *ptc-1(ok122) unc-4(e120);fog-2(q71)* females have a maturation rate of 0.56 ± 0.26 [42].
[k] Many splicing factors were identified as class II positives.

components (*inx-14* and *inx-22*) of invertebrate gap junctions [18]. To determine whether negative regulators function in the soma or the germline, we conducted RNAi analysis in an *rrf-1(null)* mutant background (Table 2). *rrf-1* encodes an RNA-dependent RNA polymerase (RdP) that is required for normal RNAi responses in many somatic cells [19] but is dispensable for germline RNAi, which employs the EGO-1 RdP [20]. Thus, an RNAi response in an *rrf-1(null)* female background is indicative of a germline function, whereas a significantly reduced response suggests gene function in the soma. As a control, we conducted *ceh-18(RNAi)* and observed elevated meiotic maturation rates in the female background, but not in *rrf-1* females (Table 2), consistent with the idea that *ceh-18* is required for normal sheath cell differentiation and function [16]. By contrast, *vab-1* functions in the germline via this test [7]. The *rrf-1* RNAi test suggests that the function of 11 genes, including *inx-14* and *inx-22*, is needed in the germline for full repression of meiotic maturation (Table 2). By contrast, the function of four genes (*goa-1*, *kin-2*, *gpb-1*, and *rpt-3*) is predominantly somatic via this test (Table 2). This observation suggests that control of meiotic maturation in *C. elegans* involves somatically acting $G\alpha_{o/i}$ and $G\alpha_s$ signaling pathways, an idea that we explore further below. The slight RNAi responses observed in *rrf-1(null)* females for *goa-1*, *kin-2*, *gpb-1*, and *rpt-3* might be due to residual somatic effects, as shown by the fact that under our conditions, *unc-22(RNAi)* produces overt muscle twitching and weak uncoordination in 5.3% of *rrf-1(null)* animals (n = 228). Nonetheless, we cannot exclude the possibility that these genes may also have some germline functions. Genetic mosaic analysis of *goa-1* and *kin-2* in a female background will be needed to test this possibility.

DAB-1, a Disabled Homolog, Functions in the VAB-1 Eph/MSP Receptor Signal Transduction Pathway for the Control of Meiotic Maturation

Previous data obtained by an in situ binding assay indicated that labeled MSP binds specifically and saturably to *C. elegans* gonads and that *vab-1(null)* gonads exhibit a significant reduction in MSP binding [7]. VAB-1 was similarly shown to be sufficient for conferring specific MSP binding to cultured mammalian cells after transient transfection [7]. These data, coupled with the finding that *vab-1* is required in the germline for full repression of meiotic maturation in the absence of MSP, led to the hypothesis that VAB-1 is one of several oocyte and sheath cell receptors that respond to the MSP signal. To more fully test the hypothesis that VAB-1 is an MSP receptor, we examined whether MSP directly binds the VAB-1 ectodomain in vitro at submicromolar concentrations. The VAB-1 ectodomain (VAB-1ECT) was expressed as a 6His-fusion in mammalian cells by its endogenous secretion signal peptide and purified from

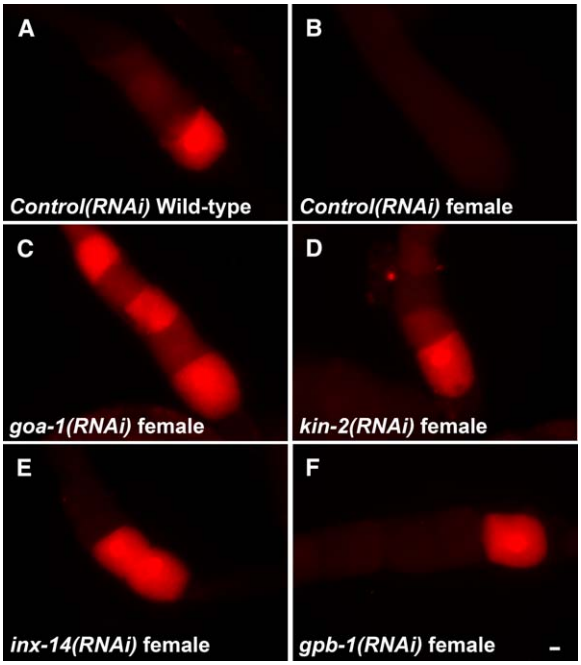

Figure 2. RNAi to Several Negative Regulators Causes MSP-Independent MAPK Activation in Oocytes

Fluorescence micrographs showing MAPK-YT staining (red) in oocytes. MAPK-YT staining is observed in the most proximal oocyte of wild-type hermaphrodites (A), but not in *fog-2(q71)* females (B). By contrast, MAPK-YT staining is observed in *fog-2(q71)* females after *goa-1(RNAi)* (C), *kin-2(RNAi)* (D), *inx-14(RNAi)* (E), and *gpb-1(RNAi)* (F). *goa-1(RNAi)* or *goa-1(null)* females exhibit expanded MAPK-YT staining to distal oocytes, though the specific pattern of relative staining intensities, such as the alternating peaks of staining in (C), can be variable. *inx-22(RNAi)* and *ptc-1(RNAi)* also result in MSP-independent MAPK activation (Table 1). Scale bar equals 10 μm.

Table 2. Parsing the Function of Negative Regulators to the Germline or Soma

RNAi	Oocyte Maturation Rate in Females[a] (N)	Oocyte Maturation Rate in *rrf-1(null)* Females[b] (N)
Control	0.17 ± 0.14 (15)	0.20 ± 0.10 (12)
ceh-18	0.44 ± 0.16 (11)[c]	0.21 ± 0.10 (10)[d]
goa-1	1.51 ± 0.20 (10)[e]	0.50 ± 0.20 (16)[f]
kin-2	1.45 ± 0.60 (12)[e]	0.54 ± 0.16 (15)[f]
gpb-1	1.03 ± 0.20 (12)[e]	0.44 ± 0.22 (16)[f]
gsa-1	0.37 ± 0.22 (13)[g]	0.21 ± 0.20 (10)[d]
rpt-3	0.51 ± 0.16 (12)[c]	0.30 ± 0.18 (12)[d]
inx-14	1.03 ± 0.22 (12)[g]	0.88 ± 0.34 (12)[f]
inx-22	0.84 ± 0.13 (12)[g]	1.00 ± 0.31 (16)[f]
ran-1	1.00 ± 0.30 (6)[g]	1.39 ± 0.32 (5)[f]
arf-1.1	0.53 ± 0.20 (9)[g]	0.45 ± 0.28 (12)[h]
ptc-1	0.63 ± 0.13 (12)[g]	0.74 ± 0.17 (14)[f]
phi-11	0.50 ± 0.20 (12)[g]	0.58 ± 0.30 (11)[f]
par-5	0.80 ± 0.29 (11)[g]	0.71 ± 0.29 (14)[f]
pqn-19	0.46 ± 0.17 (12)[g]	0.47 ± 0.14 (13)[f]
pkc-1	0.48 ± 0.16 (12)[g]	0.40 ± 0.10 (7)[f]
vav-1	0.44 ± 0.11 (11)[g]	0.48 ± 0.20 (14)[f]
dab-1	0.39 ± 0.15 (11)[g]	0.43 ± 0.10 (8)[f]

[a] Meiotic maturation rates were measured in a *fog-3(q443)* female background.
[b] Meiotic maturation rates were measured in *rrf-1(pk1417);fog-3(q443)* double mutant females.
[c] $p < 0.01$ compared to the rate after RNAi in the *rrf-1(null)* female background.
[d] $p > 0.1$ compared to the rate after control RNAi in the *rrf-1(null)* female background.
[e] $p < 0.001$ compared to the rate after RNAi in the *rrf-1(null)* female background.
[f] $p < 0.001$ compared to the rate after control RNAi in the *rrf-1(null)* female background.
[g] $p > 0.1$ compared to the rate after RNAi in the *rrf-1(null)* female background.
[h] $p < 0.01$ compared to the rate after control RNAi in the *rrf-1(null)* female background.

the culture supernatant (Figures 3A and 3B). We assessed the binding by incubating MSP with VAB-1ECT-6His and isolating the complex by immunoprecipitation with anti-MSP N-terminal-specific antibodies or by using Ni-NTA agarose. With this test, MSP and VAB-1ECT-6His exhibit direct binding (Figure 3C), with approximately 10% of the MSP bound under the binding conditions used. These data add further support to the idea that meiotic maturation is controlled in part by a VAB-1 MSP/Eph receptor signal transduction pathway.

To identify genes that play a major role in the *vab-1* pathway, we set three stringent genetic and phenotypic criteria. First, the RNAi inactivation of a *vab-1* pathway gene should derepress meiotic maturation to a similar extent as a *vab-1(null)* mutant. Second, the RNAi inactivation of a *vab-1* pathway gene should not exhibit additive or synergistic interactions with a *vab-1(null)* mutant. Finally, the RNAi inactivation of a *vab-1* pathway gene should synergize with a *ceh-18(null)* mutant. We considered the 11 genes (*inx-14*, *inx-22*, *ran-1*, *arf-1.1*, *ptc-1*, *phi-11*, *par-5*, *pqn-19*, *pkc-1*, *vav-1*, and *dab-1*), whose activity is needed in the germline for full repression of meiotic maturation, as candidates for functioning in the VAB-1 MSP/Eph receptor signal transduction pathway. Four of these genes meet these initial criteria: DAB-1, a disabled homolog, PKC-1, a protein kinase C homolog, PQN-19, a STAM homolog, and VAV-1, a Rho family guanine-nucleotide exchange factor (Figures 3D and 3E; Table S1 in the Supplemental Data available with this article online). Of these four genes, only *vav-1* was previously implicated in Eph receptor signaling by the finding that Rho family GEF Vav2 interacts with the EphA4 receptor and promotes ephrin-triggered endocytosis [21].

As a further test that *dab-1*, *vav-1*, *pkc-1*, and *pqn-19* function as part of the *vab-1* pathway, we examined the effect of null mutants and RNAi of these genes on oocyte MAPK activation in hermaphrodites (Figures 3F–3K and data not shown). *vab-1(null)* hermaphrodites exhibit an expanded pattern of MAPK activation in which MAPK-YT staining extends to distal oocytes (Figure 3G; [7]). Similarly, *dab-1(gk291* or *RNAi)*, *pkc-1(ok563* or *RNAi)*, *pqn-19(ok406* or *RNAi)*, and *vav-1(RNAi)* hermaphrodites display expanded patterns of MAPK-YT staining in oocytes (Figures 3H–3K), suggesting that these genes, like *vab-1*, function as germline negative regulators of MAPK activation. We analyzed *vav-1* only with RNAi because a *vav-1(null)* mutation is lethal [22]. Additionally, *vab-1* functions in parallel to *ceh-18* in the negative control of oocyte MAPK activation: *vab-1(null); ceh-18(null)* females show MAPK-YT staining in oocytes despite the absence of MSP [7]. By this criteria, *dab-1*, *vav-1*, *pkc-1*, and *pqn-19* behave similarly to *vab-1*, as shown by the fact that MAPK-YT staining is observed when RNAi is carried out for these genes in a *ceh-18(null)* mutant female background (Figure S1).

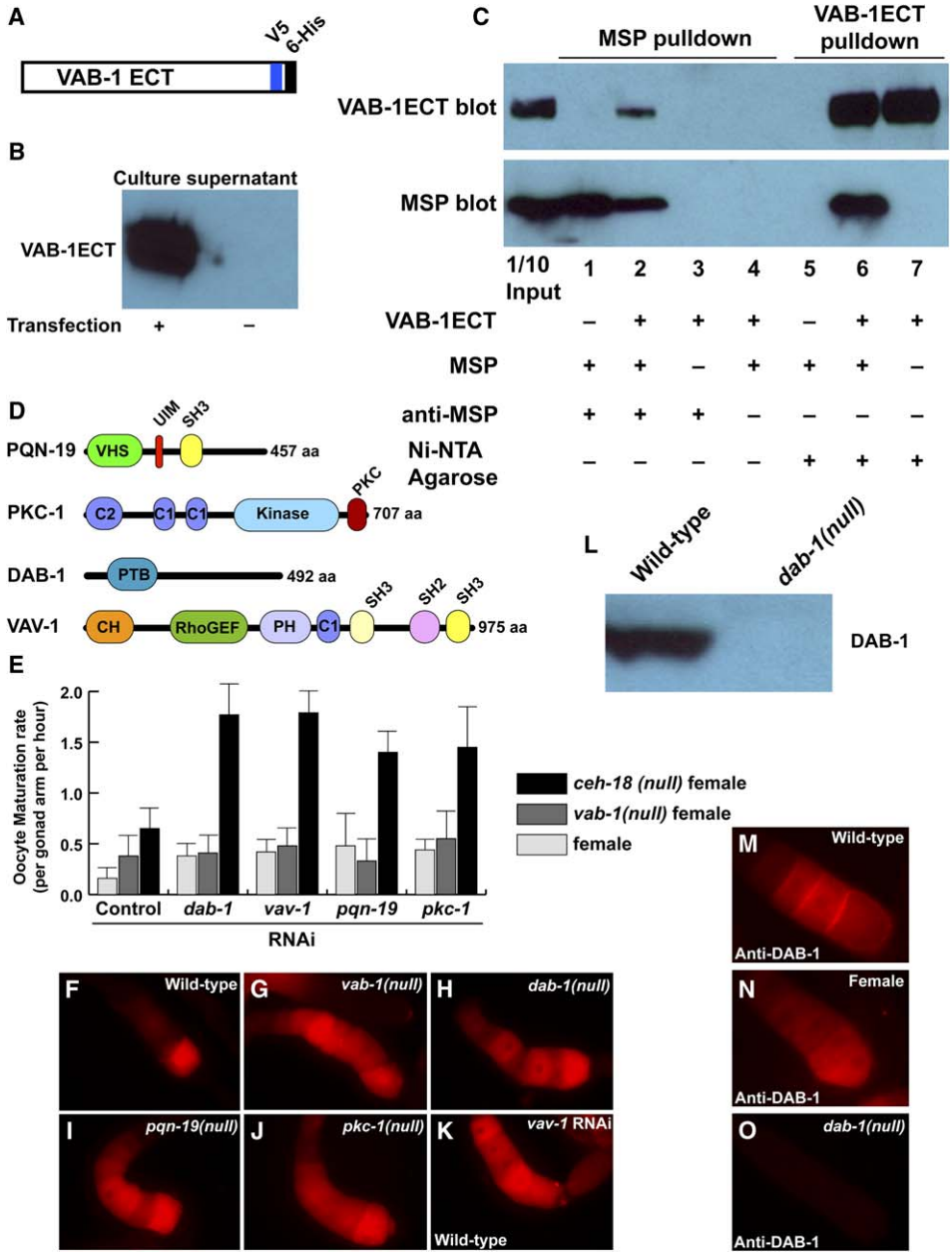

Figure 3. Genetic and Biochemical Analysis of the VAB-1 Eph/MSP Receptor Pathway

(A–C) MSP binds the VAB-1 ectodomain (VAB-1ECT).

(A) VAB-1ECT with its endogenous signal peptide and V5 and 6-His epitope tags was expressed in 293F cells.

(B) Western blot probed with V5 antibodies showing VAB-1ECT secretion into the culture medium.

(C) In vitro interaction between MSP and VAB-1ECT. Purified MSP-142 (100 nM) was incubated with partially purified VAB-1ECT (14 nM), and the complex was isolated by immunoprecipitation with MSP antibodies and protein-A Sepharose (lanes 1–4) or by Ni-NTA agarose (lanes 5–7).

(D–K) Evidence that PQN-19, PKC-1, DAB-1, and VAV-1 function with the VAB-1 Eph/MSP receptor.

(D) Organization of signaling domains in VAB-1 pathway proteins. PQN-19 contains VHS (VPS-27/Hrs/STAM), UIM (ubiquitin-interaction motif), and SH3 domains. PKC-1 contains C1, C2, kinase, and protein kinase C domains. DAB-1 contains a phosphotyrosine binding (PTB) domain. VAV-1 contains CH (calponin homology), C1, RhoGEF, SH2, and SH3 domains.

(E) Measurement of oocyte meiotic maturation rates after *dab-1*, *vav-1*, *pqn-19*, *pkc-1*, or control RNAi in *fog-2(q71)*, *vab-1(dx31);fog-2(q71)*, or *ceh-18(mg57);fog-2(q71)* female genetic backgrounds. Each of the four genes synergize with *ceh-18* but not *vab-1*. Error bars represent SD.

(F–K) Fluorescence micrographs showing MAPK-YT staining (red) in oocytes.

(F) In wild-type hermaphrodites, MSP-dependent MAPK-YT staining is observed in proximal oocytes (typically oocytes −1 through −3).

(G) MAPK-YT staining is extended to three to eight proximal oocytes in *vab-1(null)* hermaphrodites, consistent with the idea that *vab-1* is a negative regulator of meiotic maturation and MAPK activation in oocytes [7].

(H–K) Similarly, *dab-1(null)* (H), *pqn-19(null)* (I), *pkc-1(null)* (J), and *vav-1(RNAi)* hermaphrodites (K) exhibit an extended MAPK-YT staining pattern similar to that of *vab-1(null)* hermaphrodites (G).

(L–O) DAB-1 is expressed in oocytes. Western blot detection of DAB-1 (53 kDa) in the wild-type, but not *dab-1(gk291)* (L), hermaphrodites. Fluorescent detection of DAB-1 in oocytes from wild-type hermaphrodites (M) and *fog-2(q71)* females (N), but not *dab-1(gk291)* hermaphrodites (O). DAB-1 is cortically enriched when sperm are present.

Because strong conclusions regarding genetic pathways are not possible without phenotypic analysis of null mutations, we analyzed meiotic maturation phenotypes in deletions alleles of *dab-1*, *pkc-1*, and *pqn-19*, which are predicted to significantly reduce or eliminate gene function. To further explore the involvement of *dab-1* disabled, we analyzed meiotic maturation rates in females homozygous for a *dab-1* null mutation, *gk291*, which deletes exons two and three, including the phosphotyrosine binding domain [23, 24]. These *dab-1(gk291)* null mutant females exhibit increased meiotic maturation rates (0.42 ± 0.14 [n = 22]) compared to normal females (0.16 ± 0.1 [n = 17], $p < 0.001$). This increase in meiotic maturation rate is similar to that observed in *vab-1(null)* mutant females (0.38 ± 0.25 [n = 18]). Further, *vab-1(null)dab-1(null)* double mutant females exhibit a meiotic maturation rate of 0.43 ± 0.19 (n = 22), consistent with the idea that these two genes function in a common pathway. Additional evidence supporting the idea that *dab-1* functions in an oocyte pathway comes from the finding that DAB-1 protein localizes to the oocyte cytoplasm and is enriched at the oocyte cell cortex between oocytes (Figure 3M) in a pattern similar to VAB-1::GFP [7]. Strikingly, DAB-1 protein localization is altered in the absence of sperm, no longer exhibiting cortical enrichment between oocytes (Figure 3N). Recent data indicate that DAB-1 physically interacts with the VAB-1 intracellular domain in vitro (H.C. and D.G., unpublished results).

In contrast to *dab-1*, we did not observe elevated meiotic maturation rates in *pqn-19(ok406)fog-3(q443)* females, which displayed rates (0.10 ± 0.15; n = 22) similar to unmated female controls. Likewise, meiotic maturation rates in *pkc-1(ok563);fog-3(q443)* females, though slightly elevated (0.18 ± 0.20; n = 24), were significantly lower than those of *vab-1* females ($p < 0.001$). Thus, for *pqn-19* and *pkc-1*, the analysis of meiotic maturation rates in a female background led to a different conclusion from the RNAi and MAPK activation studies described above. To reconcile this discrepancy, we analyzed oocyte meiotic maturation by time-lapse videomicroscopy and noticed that *pqn-19(ok406)* and *pkc-1(ok563)* hermaphrodites exhibited an incompletely penetrant (~33%) delay in nuclear envelope breakdown during oocyte meiotic maturation. We made the same observation in *dab-1(gk291)* hermaphrodites, and similar data were published for *vav-1(null)* mutant hermaphrodites for which the lethal pharyngeal defects were transgenically rescued [22]. Since this delay in nuclear envelope breakdown is not observed in *vab-1(null)* mutants, we conclude that *dab-1*, *pkc-1*, *pqn-19*, and *vav-1* may also have redundant functions as positive regulators of meiotic maturation, through *vab-1*-independent pathways.

Necessity and Sufficiency of Somatic $G\alpha_{o/i}$ Signaling in the Control of Meiotic Maturation and Oocyte MAPK Activation

The strongest negative regulator of meiotic maturation identified in the RNAi screen is *goa-1*, which encodes a heterotrimeric $G_{o/i}\alpha$ protein previously shown to regulate locomotion, egg-laying, and male mating behaviors [25, 26]. *goa-1(RNAi)* in a female background triggers meiotic maturation and MAPK activation in oocytes despite the absence of MSP (Table 1 and Figure 2C). Extending these RNAi results via genetics, we found that *goa-1(sa734)* null mutant females exhibited significantly higher meiotic maturation rates than control females and they showed MSP-independent MAPK activation in oocytes (Table 3, compare lines 4 and 2, and Figure 4C, $p < 0.001$). The *goa-1(n1134)* reduction-of-function (rf) allele behaved similarly (Table 3, compare lines 6 and 2, and Figure 4E, $p < 0.001$). Meiotic maturation rates were lower in *goa-1(sa734)* null mutant females compared to *goa-1(RNAi)* females; however, this is most likely because the *goa-1(sa734)* females appeared starved and produced fewer oocytes. Consistent with this interpretation, *goa-1(n1134rf)* females were healthier and exhibited higher meiotic maturation rates than null mutant females, and *goa-1(sa734)* hermaphrodites had lower rates than the wild-type (Table 3, compare lines 1–7). Time-lapse videomicroscopy of meiotic maturation and ovulation in *goa-1(n1134)fog-3(q443)* (n = 8) and *goa-1(RNAi);fog-2(q71)* (n = 8) females indicated that nuclear envelope breakdown, cortical cytoskeletal rearrangement, and ovulation all occurred normally despite the absence of MSP.

MSP-dependent MAPK activation and meiotic maturation require the downstream action of OMA-1 and OMA-2, two TIS-11 zinc finger proteins expressed in the germline [11]. No MAPK activation or meiotic maturation is observed in *goa-1(sa734);oma-1(RNAi);oma-2(RNAi)* or *goa-1(n1134);oma-1(RNAi);oma-2(RNAi)* hermaphrodites and females (Figure 4D, Table 3, lines 9 and 10, and Figure S2). Thus, $G\alpha_{o/i}$ likely functions upstream or in parallel with OMA-1/OMA-2 to repress meiotic maturation in the absence of the MSP signal, with the caveat that RNAi is not necessarily equivalent to null mutations in genetic epistasis experiments. To test whether GOA-1 activity is sufficient to repress meiotic maturation, we measured meiotic maturation rates in hermaphrodites expressing constitutively activated GOA-1(Q205L) under control of the *goa-1* promoter [25] and observed that meiotic maturation rates were reduced by 70% despite the presence of MSP (Table 3, line 11). These results suggest that *goa-1* activity is needed to fully repress meiotic maturation in the absence of MSP and that it is also sufficient to partially repress meiotic maturation in the presence of MSP. Since transgenes are ordinarily silenced in the germline [27], this data constitutes a further line of evidence suggesting that *goa-1* functions in the soma.

The analysis of *goa-1(RNAi)* in an *rrf-1* mutant female background described above suggests that *goa-1* functions in the somatic control of oocyte meiotic maturation (Table 2). Since *goa-1* is maternally required for positioning mitotic spindles in embryonic blastomeres [28], we examined the expression of GOA-1 in dissected gonads of hermaphrodites and females via specific antibodies (Figure 5). In hermaphrodites, we observed cytoplasmic staining in oocytes as well as staining that appeared to be in the surrounding sheath (Figure 5A). No staining was observed in *goa-1(sa734)* null mutants or after *goa-1 (RNAi)*, confirming the specificity of the antibodies (Figure 5C and data not shown). In dissected gonads from females, we observed cortical enrichment of GOA-1 between oocytes as well as staining that appeared to be in the sheath (Figure 5B). Since sheath cell and oocyte

Table 3. Genetic Analysis of G Protein Signaling

	Genotype[a]	Sperm (yes/no)	Oocyte Maturation Rate[b]	MAPK Activation[c]
1.	Wild-type hermaphrodite	yes	2.50 ± 0.41 (16)	on
2.	*fog-3(q443)* unmated	no	0.17 ± 0.14 (15)	off
3.	*fog-3(q443)* mated	yes	2.29 ± 0.43 (20)	on
4.	*goa-1(sa734) fog-3(q443)* female	no	0.47 ± 0.17 (30)	on
5.	*goa-1(sa734)*hermaphrodite	yes	1.23 ± 0.18 (15)	on
6.	*goa-1(n1134)fog-3(q443)* female	no	1.06 ± 0.25 (16)	on
7.	*goa-1(n1134)* hermaphrodite	yes	2.55 ± 0.40 (18)	on
8.	*oma-1(RNAi);oma-2(RNAi)* hermaphrodite	yes	0.06 ± 0.07 (13)	off
9.	*oma-1(RNAi);oma-2* (RNAi); *goa-1(sa734)fog-3(q443)* female	no	0.00 ± 0.00 (13)	off
10.	*oma-1(RNAi);oma-2* (RNAi); *goa-1(sa734)* hermaphrodite	yes	0.00 ± 0.00 (17)	off
11.	*goa-1(gf)* hermaphrodite[d]	yes	0.76 ± 0.62 (19)	on
12.	*kin-2(ce179* rf*);fog-3(q443)* female	no	0.64 ± 0.23 (14)	on
13.	*gsa-1* (RNAi) wild-type hermaphrodite	yes	0.30 ± 0.11 (17)	off
14.	Control (RNAi); *vab-1(dx31)* hermaphrodite	yes	2.44 ± 0.60 (15)	on
15.	*gsa-1* (RNAi); *vab-1(dx31)* hermaphrodite	yes	0.20 ± 0.17 (15)	off
16.	Control (RNAi); *kin-2(ce179* rf*)* hermaphrodite	yes	1.92 ± 0.59 (12)	on
17.	*gsa-1* (RNAi); *kin-2(ce179* rf*)* hermaphrodite	yes	1.78 ± 0.46 (15)	on
18.	*gsa-1(ce81* gf*) fog-3(q443)* female	no	0.38 ± 0.14 (12)	on
19.	*gsa-1(ce94* gf*) fog-3(q443)* female	no	0.44 ± 0.13 (13)	on
20.	*gsa-1(pk75)/+* hermaphrodite	yes	1.60 ± 0.40 (10)	on
21.	Control (RNAi); *rrf-1(pk1417)* hermaphrodite	yes	2.39 ± 0.50 (12)	on
22.	*gsa-1* (RNAi); *rrf-1(pk1417)* hermaphrodite	yes	2.06 ± 0.40 (15)	on
23.	*gsa-1* (RNAi); *goa-1(sa734)* hermaphrodite	yes	0.14 ± 0.10 (16)	off
24.	*gsa-1* (RNAi); *goa-1(n1134)* hermaphrodite	yes	0.34 ± 0.20 (14)	off
25.	*gsa-1* (RNAi); *goa-1(sa734)fog-3(q443)*	no	0.16 ± 0.10 (10)	off
26.	Control (RNAi); *ceh-18 (mg57)* hermaphrodite	yes	1.76 ± 0.44 (15)	on
27.	*gsa-1* (RNAi); *ceh-18(mg57)* hermaphrodite[e]	yes	1.78 ± 0.36 (16)	on
28.	*oma-1;oma-2* (RNAi); *gsa-1(ce94* gf*)fog-3(q443)* female	no	0.00 ± 0.00 (12)	off

[a] Genotypes utilized null mutations unless where indicated by "gf" or "rf" for gain-of-function and reduction-of-function mutations, respectively. The position and morphology of sheath cell nuclei were unaffected by RNAi of *gsa-1*, *kin-2*, or *goa-1*, or in mutants of these genes.
[b] Meiotic maturation rates were measured in 2-day-old adult animals.
[c] MAPK activation was scored as described above, with "on" denoting strong staining in proximal oocytes and "off" denoting an absence of staining.
[d] The PS1493 transgenic strain that expresses constitutively-activated GOA-1($G\alpha_o$QL) under the control of *goa-1* promoter was used [25].
[e] *ceh-18* mutant sheath cells respond to RNAi, as *gfp(RNAi)* could silence *lim-7::gfp* expression in a *ceh-18(mg57)* mutant background.

plasma membranes are in close apposition and the sheath cells are extremely thin (~0.2 μm; [17]), we needed a way to visualize GOA-1 expression in sheath cells separately from oocytes. Thus, we reduced the expression of GOA-1 in the germline by performing *goa-1* RNAi on *rrf-1* females, and we stained the dissected gonads with anti-GOA-1 antibody. In these *goa-1(RNAi); rrf-1* female gonads, cortical GOA-1 staining between oocytes is significantly reduced through medial focal planes, yet staining persists in the thin layer surrounding oocytes, consistent with sheath cell expression (Figures 5D and 5E). The gonadal sheath cells insert finger-like projections between oocytes that can be resolved only by electron microscopy [17]. In these *goa-1(RNAi);rrf-1* female gonads, punctate staining is observed between oocytes mainly in superficial focal planes, suggesting that GOA-1 may be present in the sheath cell processes (Figure 5E). We stained dissected gonads from these *goa-1(RNAi);rrf-1* mutant females with MAPK-YT antibodies and observed an absence of MAPK activation in the proximal gonad, suggesting that *goa-1* activity might be sufficient in the soma to negatively regulate MAPK activation in oocytes (Figure 5F). Since the sheath cells mediate the *ceh-18*-dependent inhibition of MAPK activation and meiotic maturation, which in turn is antagonized by MSP [7], we tested whether *ceh-18* and *goa-1* genetically interact. The high meiotic maturation rate (1.67 ± 0.38) observed after *goa-1(RNAi)* in a *fog-2(q71)* background depends on *ceh-18(+)* function because *goa-1(RNAi)* in a *ceh-18(null);fog-2(q71)* background results in a lower meiotic maturation rate (0.92 ± 0.30; see Table S1). This result is consistent with a model in which *goa-1* functions in the sheath cell control of meiotic maturation or acts in parallel to *ceh-18*. A role for *goa-1* in the soma is additionally suggested by the observation that the basal sheath cell contraction rate is elevated in *goa-1(n1134)fog-3(q443)* and *goa-1(RNAi); fog-2(q71)* females (data not shown). Genetic epistasis analysis between $G\alpha_{o/i}$, $G\alpha_s$, *ceh-18*, and innexins (see below) further supports a role for *goa-1* in regulating sheath/oocyte communication.

A Somatic $G\alpha_s$ Signaling Pathway Is Necessary and Sufficient to Promote Meiotic Maturation

In canonical $G\alpha_s$ signaling, activated $G\alpha_s$ stimulates adenylyl cyclase resulting in production of cAMP, which binds the regulatory subunit of cAMP-dependent PKA, thereby releasing the active catalytic subunit [29]. We identified *kin-2*, which encodes the regulatory subunit of cAMP-activated protein kinase, as a strong negative regulator of meiotic maturation and found that *kin-2* functions somatically to inhibit meiotic maturation and MAPK activation in oocytes (Tables 1 and 2, Figure 2D, and data not shown). Time-lapse videomicroscopic analysis of *kin-2(RNAi)* in *fog-2(q71)* females (n = 4) indicates that meiotic maturation occurs normally and that basal sheath cell contractions are elevated despite the absence of MSP. Consistent with the RNAi results,

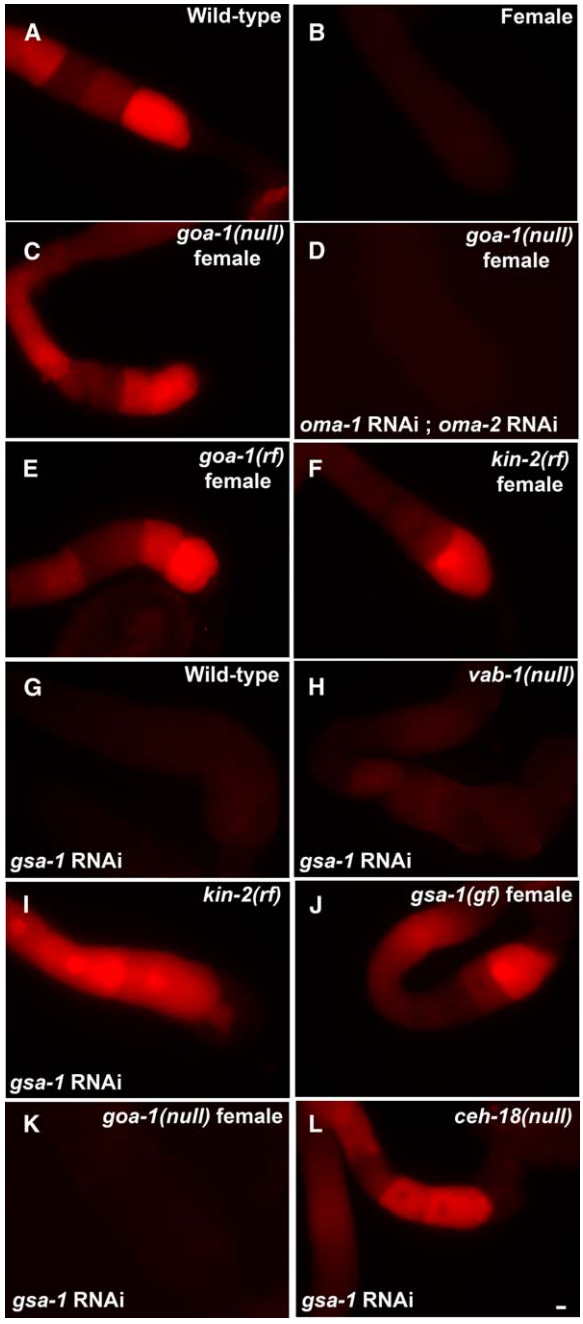

Figure 4. $G\alpha_{o/i}$ and $G\alpha_s$ Signaling Antagonistically Regulate Oocyte MAPK Activation

Fluorescent micrographs showing MAPK-YT staining (red) in oocytes after the indicated genetic or RNAi perturbations of the $G\alpha_{o/i}$ and $G\alpha_s$ pathways in hermaphrodites (A, G, H, I, L) or females (B–F, J, K). Scale bars equal 10 μm.

*kin-2(ce179*rf*)* females exhibit increased meiotic maturation rates compared to control females (Table 3, compare lines 12 and 2, $p < 0.001$), and they display MAPK activation in proximal oocytes (Figure 4F). These results predict that *gsa-1*, which encodes cAMP-stimulatory $G\alpha_s$, should function to promote meiotic maturation. Surprisingly, our RNAi screen identified *gsa-1* as a weak negative regulator of meiotic maturation in the absence of sperm (Table 1).

To resolve this paradox, we reasoned that *gsa-1* might have an MSP-dependent function in promoting meiotic maturation through the canonical pathway and a weak MSP-independent function in inhibiting meiotic maturation through a noncanonical pathway. Importantly, our RNAi screen could not reveal a positive role for *gsa-1* because it was conducted in the absence of the MSP signal. Consistent with this hypothesis, *gsa-1(RNAi)* in a wild-type hermaphrodite background results in a 90% reduction in the meiotic maturation rate (Table 3, compare lines 13 and 1) and strikingly blocks MAPK activation in proximal oocytes (Figure 4G). Further, *gsa-1(RNAi)* can block meiotic maturation and MAPK activation in the *vab-1(null)* mutant hermaphrodite background where MAPK activation is ordinarily expanded to distal oocytes (Table 3, compare lines 14 and 15, and Figure 4H). $G\alpha_s$ signals through the canonical pathway to promote meiotic maturation because *gsa-1(RNAi); kin-2(ce179*rf*)* hermaphrodites undergo meiotic maturation at 93% of the rate of *kin-2(ce179*rf*)* hermaphrodites treated with control RNAi, and their proximal oocytes contain activated MAPK (Table 3, compare lines 16 and 17, and Figure 4I). Since the *gsa-1(pk75)* null mutant is a larval lethal [30], we examined *gsa-1 (pk75)/+* heterozygous hermaphrodites and observed a significant 36% reduction in the meiotic maturation rates (Table 3, compare lines 20 and 1, $p < 0.001$). We also noted that *gsa-1(pk75)/+* hermaphrodites moved slowly and were slightly egg-laying defective but were otherwise healthy and well-fed, suggesting that *gsa-1* is haploinsufficient for multiple phenotypes. We performed *gsa-1(RNAi)* in *rrf-1(null)* hermaphrodites and observed similar meiotic maturation rates and MAPK activation as *rrf-1(null)* hermaphrodites treated with control RNAi (Table 3, compare lines 21 and 22), suggesting that *gsa-1* function may be sufficient in the soma and dispensable in the germline. Consistent with these data, we found that extrachromosomal arrays bearing transcriptional and translational *gsa-1::gfp* reporter constructs are expressed in the somatic sheath cells (data not shown).

To examine whether *gsa-1* activity is sufficient to promote meiotic maturation, we examined two dominant gain-of-function (gf) *gsa-1* alleles, *ce94*gf and *ce81*gf, which are predicted to stabilize the GTP bound form of $G\alpha_s$ through G45R and R182C substitutions, respectively [31]. *gsa-1(ce94*gf*)* and *gsa-1(ce81*gf*)* females display elevated meiotic maturation rates and MAPK activation in oocytes despite the absence of MSP (Table 3, compare lines 18, 19, and 2, and Figure 4J). No MAPK activation or meiotic maturation was observed in *oma-1(RNAi);oma-2(RNAi);gsa-1(ce94*gf*)* hermaphrodites or females (Table 3, compare lines 19 and 28, and data not shown), suggesting that $G\alpha_s$ either is an upstream regulator or functions in parallel. These data are consistent with a model in which somatic *gsa-1* activity is necessary and sufficient for promoting meiotic maturation and MAPK activation in oocytes.

$G\alpha_{o/i}$ Antagonizes $G\alpha_s$ Signaling to Repress Meiotic Maturation in the Absence of MSP

Since $G\alpha_{o/i}$ activity is required for repressing meiotic maturation in the absence of MSP and $G\alpha_s$ signaling is necessary and sufficient to promote meiotic maturation, we asked whether *goa-1* negatively regulates *gsa-1* in

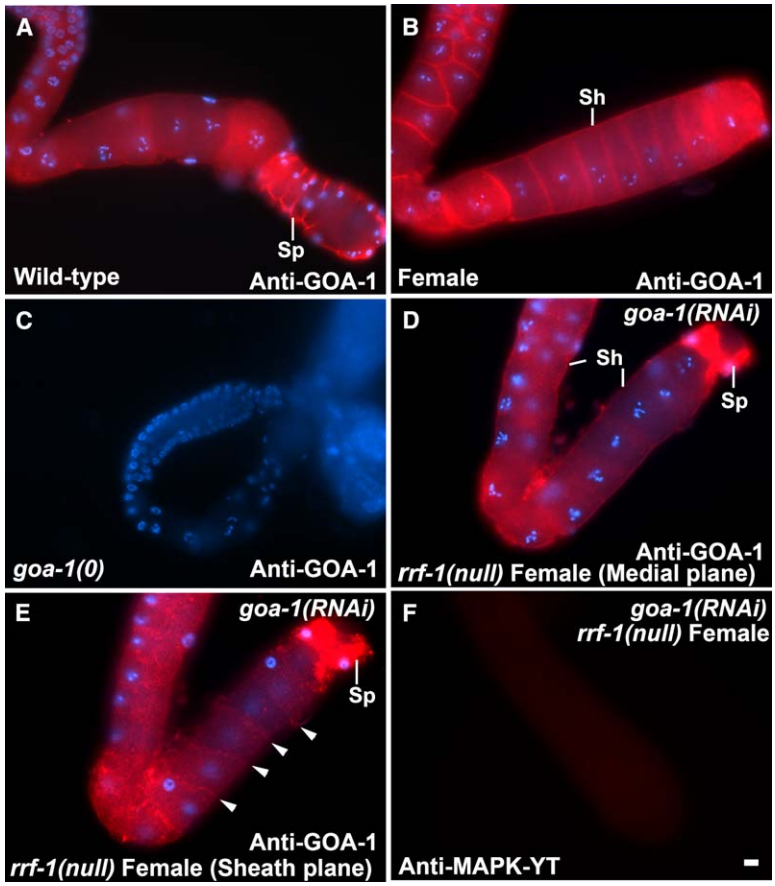

Figure 5. Expression of GOA-1 in the Soma Is Sufficient to Inhibit MAPK Activation in Oocytes

(A–E) Fluorescent micrographs of dissected gonads stained for GOA-1 (red) and DNA (blue). GOA-1 is expressed in sheath cells, oocytes, and spermathecal cells of wild-type hermaphrodites (A) and *fog-2(q71)* (B) and *fog-3(q443)* (not shown) females. In females, GOA-1 is cortically enriched between oocytes (B). No GOA-1 staining is observed in *goa-1(sa734)* mutants (C). GOA-1 staining in oocytes is significantly reduced after *goa-1(RNAi)* in an *rrf-1(null)fog-3(q443)* mutant female background (D). Note, GOA-1 staining between oocytes is reduced in the medial focal plane, comparing (D) and (B), yet staining in the sheath (Sh) and spermatheca (Sp) persists. GOA-1 staining also persists when viewed in superficial focal planes (E) in *goa-1(RNAi);rrf-1(null)fog-3(q443)* females with punctate staining possibly corresponding to the sheath cell processes (arrowheads).

(F) Fluorescence micrograph of MAPK-YT staining after *goa-1(RNAi)* in a *rrf-1(null)fog-3(q443)* mutant female background. MAPK-YT staining is not observed, suggesting that GOA-1 expression in the soma (D and E) is sufficient to repress MAPK activation in oocytes.

analogy to the regulation of the $G\alpha_q$ *egl-30* pathway by *goa-1* in neurons [28]. We performed *gsa-1(RNAi)* on *goa-1(sa734)* and *goa-1(n1134)* hermaphrodites and females and observed low meiotic maturation rates and an absence of MAPK activation in proximal oocytes despite the presence of MSP (Table 3, lines 23–25, Figure 4K). This result suggests that the important function of $G\alpha_{o/i}$ in blocking meiotic maturation when sperm are unavailable for fertilization operates via the control of $G\alpha_s$ signaling or through the regulation of a parallel pathway.

A key clue of how *gsa-1* might promote meiotic maturation comes from the observation that *gsa-1(RNAi); ceh-18(null)* hermaphrodites exhibit normal meiotic maturation rates and show MAPK activation in oocytes (Table 3, compare lines 26 and 27, Figure 4L). How might *gsa-1*'s function to promote meiotic maturation become dispensable in the absence of *ceh-18* activity? In *ceh-18(null)* mutants, sheath cells and oocytes are not in close apposition and sheath/oocyte gap junctions are rare or absent [16, 17]. Our RNAi screen identified *inx-14* and *inx-22* as germline negative regulators of meiotic maturation and MAPK activation in the absence of sperm (Tables 1 and 2, Figure 2D). Since oocytes have been observed to form gap junctions only with sheath cells [17], *inx-14* and *inx-22* likely encode oocyte components of sheath/oocyte gap junctions. Since sheath/oocyte gap junctions must be lost when oocytes lose contact with sheath cells during ovulation, we considered the possibility that $G\alpha_s$ signaling promotes meiotic maturation in part by destabilizing inhibitory sheath/oocyte gap junctions. To test this possibility, we conducted *gsa-1;inx-14* double RNAi experiments in wild-type and *inx-22(tm1661)* backgrounds under conditions in which we could verify that both RNAi treatments were effective (Table S2). Whereas meiotic maturation was blocked after *gsa-1(RNAi)* in the wild-type and *inx-22(tm1661)* backgrounds, meiotic maturation occurred normally after *gsa-1(RNAi);inx-14(RNAi)* in the *inx-22(tm1661)* background. Importantly, meiotic maturation was blocked after *gsa-1(RNAi) inx-14(RNAi)* in the wild-type. This result suggests that reduction of both *inx-14* and *inx-22* function is needed to bypass the requirement for *gsa-1* for normal meiotic maturation. Taken together, these data suggest that $G\alpha_s$ signaling may promote meiotic maturation in part by affecting the synthesis or stability of sheath/oocyte gap junctions, or through action in a parallel pathway (Figure 6). While further studies will be needed to test this model at cell biological and ultrastructural levels, these data are consistent with studies in several systems, which show that G protein signaling can promote the assembly or disassembly of gap junctions [32–34].

Similarities and Differences in Meiotic Maturation Signaling in *C. elegans* and Mammals

Previous studies demonstrated that somatic and germline regulatory pathways work in concert to control oocyte meiotic maturation [2, 13]. Here we employed a genome-wide RNAi screen to define new regulators of

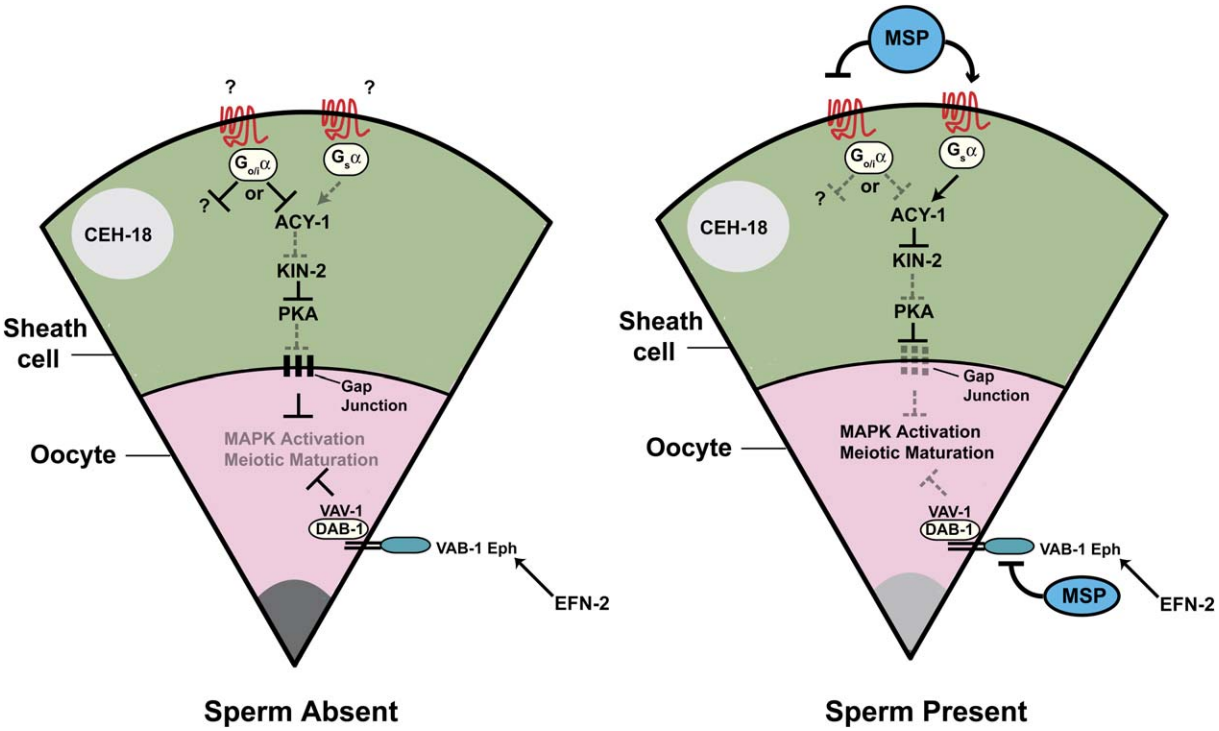

Figure 6. A Model for the Parallel Control of Meiotic Maturation in *C. elegans* by Antagonistic G Protein Signaling from the Soma and an Oocyte MSP/Eph Receptor Pathway

The germline and soma meiotic maturation control network is depicted in two states, according to whether the MSP signal is absent (left) or present (right). $G\alpha_{o/i}$ negatively regulates meiotic maturation and oocyte MAPK activation and antagonizes a $G\alpha_s$ pathway that promotes maturation. The $G\alpha_s$ pathway is drawn showing the involvement of the regulatory subunit (KIN-2) of cyclic-AMP-dependent protein kinase A (PKA) and adenylate cyclase (ACY). Genetic evidence is presented here for the involvement of *kin-2*, but which of the four *acy* genes participates in this regulation is not yet clear; however, the *acy-1(ce2gf)* allele has a weak effect (J.A.G. and D.G., unpublished results). Unidentified sheath cell GPCRs are proposed to receive the MSP signal in parallel to VAB-1 on the oocyte, such that GPCRs coupled to $G\alpha_{o/i}$ are antagonized by MSP, whereas $G\alpha_s$-coupled receptors are stimulated by MSP. The $G\alpha_s$ pathway is proposed to directly destabilize the inhibitory sheath/oocyte gap junctions, but a parallel function is equally consistent with current genetic data. The CEH-18 POU-homeoprotein localizes to sheath cell nuclei where it functions in the control of sheath cell differentiation and function, in part, by directly or indirectly affecting the assembly of sheath/oocyte gap junctions. DAB-1 and VAV-1 function in the VAB-1 MSP/Eph receptor pathway in the germline.

oocyte meiotic maturation in *C. elegans*. The set of regulators defined in this screen comprises 17 highly conserved proteins (Table 1), which mediate meiotic maturation signaling functions in the somatic gonadal sheath cells or oocytes.

The RNAi screen identified four genes (*dab-1*, *vav-1*, *pqn-19*, and *pkc-1*) satisfying multiple genetic criteria expected of genes functioning in the *vab-1* Eph receptor pathway (Figure 6). *vab-1* was previously shown to be necessary for complete MSP binding to gonads via an in situ binding assay and also to be sufficient to confer specific MSP binding activity to cultured mammalian cells [7]. Data presented here showing that the VAB-1 ectodomain directly binds MSP, taken together with functional genetic analyses, provide strong evidence that MSP promotes meiotic maturation in part by antagonizing an Eph receptor signaling pathway in oocytes as proposed [7]. Recently, it was suggested that VAB-1 may switch from a negative regulator to a redundant positive regulator of meiotic maturation upon binding MSP [14]. Consistent with the possibility that negative regulators of meiotic maturation may also have redundant activating functions, we found that mutations in *dab-1*, *pqn-19*, and *pkc-1* confer an incompletely penetrant delay in nuclear envelope breakdown during meiotic maturation, and a similar observation was made previously for *vav-1* [22]. The maturation-promoting redundant functions of *dab-1*, *pqn-19*, *pkc-1*, and *vav-1* are likely through a *vab-1*-independent pathway because *vab-1(null)* mutations do not exhibit delays in nuclear envelope breakdown. The mechanisms by which the *vab-1* pathway represses meiotic maturation and MAPK activation in oocytes in the absence of MSP will take additional work to decipher. Nonetheless, the conserved *vab-1* pathway genes described here are likely to mediate analogous signaling functions in mammals. In fact, a recent study of Eph receptor signaling during axonal guidance in mammals found a critical role for a homolog of VAV-1, the Rho family guanine nucleotide exchange factor Vav2 [21].

Our findings suggest that antagonistic $G\alpha_s$ and $G\alpha_{o/i}$ protein signaling pathways play a predominant role in mediating the control of meiotic maturation likely by the gonadal sheath cells. $G\alpha_{o/i}$ defines a negatively acting pathway, whereas $G\alpha_s$ defines a positively acting pathway (Figure 6). In part, $G\alpha_s$ may promote meiotic maturation by antagonizing inhibitory sheath/oocyte gap-junctional communication. Since *gsa-1(RNAi)* is epistatic to *goa-1(null)* mutations, $G\alpha_{o/i}$ signaling might inhibit the $G\alpha_s$ pathway at some level, perhaps by

interfering with the activation of $G\alpha_s$ or possibly through inhibition of adenlyate cyclase. Alternatively, $G\alpha_s$ and $G\alpha_{o/i}$ may converge at some point far downstream, in effect, defining parallel regulatory inputs. Nonetheless, these results lead us to suggest that the gonadal sheath cells have the dual function of inhibiting meiotic maturation in the absence of MSP and promoting it in the presence of MSP. We speculate that unidentified MSP receptors [7] may be G protein-coupled receptors (GPCRs) expressed in the gonadal sheath cells (Figure 6). Our results illustrate that the control of meiotic diapause in *C. elegans* involves multiple layers of control involving both the soma and the germline. This multitiered control mechanism may be important for tightly repressing meiotic maturation when sperm are unavailable, while also enabling graded responses that match the meiotic maturation rate to the number of sperm in the reproductive tract.

Our findings highlight interesting parallels and underscore fundamental differences between the control of meiotic maturation in *C. elegans* and mammals. In both cases, the somatic gonad may function to promote or inhibit meiotic progression depending on the hormonal status of the organism. For example, the removal of oocytes from large antral follicles causes meiotic resumption in most mammals [15, 35]. At the same time, luteinizing hormone (LH) receptor signaling in the mural granulosa cell compartment of the ovary promotes meiotic maturation in part through the triggered release of EGF-like ligands that induce meiotic resumption [36]. The LH receptor is $G\alpha_s$-coupled GPCR, and thus in mammals and *C. elegans*, $G\alpha_s$ signaling in somatic cells has a meiotic maturation-promoting function. In contrast, $G\alpha_s$ signaling within oocytes involving the GPR3 orphan GPCR plays a critical role in promoting meiotic arrest in mice [37–39]. In mammals, these multiple levels of control, involving the somatic gonad and the germline, may serve to maintain oocyte homeostasis during the prolonged meiotic arrest, while at the same time integrating the behaviors of the somatic gonad and the germline so as to coordinate nuclear and cytoplasmic meiotic maturation events with ovulation. In humans, defects in female meiosis I represent the leading cause of congenital birth defects and miscarriage, and the frequency of these meiotic errors increases with maternal age [3]. In the aging ovarian environment, defective hormonal signaling responses may be a factor underlying the high rate of aneuploidy [40, 41]. Since *goa-1(null)* mutations cause nondisjunction during female meiosis (J.A.G. and D.G., unpublished results), signaling defects may also contribute to aneuploidy in *C. elegans*. The conserved regulatory genes described here are therefore expected to facilitate an understanding of how perturbations in hormonal signaling might contribute to aneuploidy.

Conclusions

We defined germline and somatic signaling pathways that maintain meiotic arrest of *C. elegans* oocytes in the absence of the MSP signal. The underlying logic of meiotic diapause control in *C. elegans* and mammals is remarkably similar—both utilize multiple layers of control involving the soma and the germline, and G protein signaling can promote or repress meiotic maturation depending on cellular context.

Supplemental Data

Supplemental Data include two figures, two tables, and Supplemental Experimental Procedures and are available with this article online at http://www.current-biology.com/cgi/content/full/16/13/1257/DC1/.

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